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SSR diversity of vegetable soybean [*Glycine max* (L.) Merr.]

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Abstract

Edamame [*Glycine max* (L.) Merr.] is a type of soybean selected for fresh or frozen vegetable use at an immature stage. Since edamame has a similar protein content, milder flavor, nuttier texture, and is easier to cook when compared to grain soybean, it is being promoted as a new vegetable for global consumption. Global production will require breeding programs for local adaptation; however, limited research has been published on genetic diversity of edamame varieties for the assessment of genetic resources. Simple sequence repeats (SSRs) were used to study the genetic diversity among 130 accessions, including edamame cultivars and landraces from Japan, China and the US, and also the new breeding lines in the US. Although it is assumed that elite edamame cultivars would have narrow genetic diversity, seventeen SSRs detected polymorphism to distinguish 99 of the 130 accessions. The cluster analysis generated nine clusters and 18 outliers. Genetic diversity within Japanese edamame was lower than that within Chinese vegetable soybean accessions (maodou), even though only 10 Chinese maodou were analyzed compared to 107 Japanese edamame. Cluster analysis revealed that the patterns of SSR diversity in edamame can generally distinguish maturity classes and testa color. We concluded that Japanese edamame have a narrow genetic base different from others and that SSRs can describe the patterns of genetic diversity among the elite vegetable soybean.

Introduction

Edamame is the Japanese name for a special type of immature soybean [*Glycine max* (L.) Merr.] that is consumed as a vegetable or snack. It is called vegetable soybean, green soybean or edible soybean in North America and maodou in China. Like wasabi and other food traditions imported from Japan, the name edamame has been introduced into the American English vernacular. Edamame is harvested at the immature R6 (fully expanded seed) stage (Fehr and Caviness 1977) and, like common grain soybean, are rich in protein and highly nutritious, but they are nonetheless characteristically different from grain soybean. Dry edamame seed is larger (usually over 30 g/100 seeds), has a higher soluble sugar content and a lower number of chemical components associated with negative flavors than grain soybean, reviewed by Konovsky et al. (1994). Although edamame is a minor crop in

the world, it is quite popular in East Asia, especially in Japan, China, Korea and Taiwan. Japan is the largest market. In other locations edamame is also being recognized as a highly nutritious food and/or as a valuable product for export to Japan (Shurtleff and Lumpkin 2001). Imported and domestic frozen edamame can be found in Asian markets and major grocery stores in the US. In 1999, the volume of edamame sold in the Japanese wholesale vegetable market was 35,921 tons, worth US $140 million (MAFF 2000). Japan imports 30% of its fresh edamame and majority parts of the frozen product from China, Thailand, and Taiwan (Takahashi 1991), although local fresh edamame is believed to be superior in flavor and fetches a premium price in Japan.

The breeding of edamame cultivars is mostly a private sector activity in Japan. There are many landraces from common ancestors that have been segregated by farmers' selection for local environments and farmers' preferences (Kohno 1988). The same edamame cultivars sometimes have different commercial names (Shimizu 1977), perhaps due to sequential local distributions. Edamame breeding has also progressed in Southeast Asia (Wasee et al. 1992), Brazil (Yokomizo and Vello 1998), Puerto Rico (Camancho-Chacón et al. 1999) and Australia (Nguyen 1997).

The narrowness of the genetic base of US grain soybean cultivars by pedigree was detected by parentage analysis (Gizlice et al. 1994) and genetic markers (e.g. Narvel et al. 2000). Public Japanese soybean cultivars originated from Japanese ancestors had also low parentage with exotic ancestors (Zhou et al. 2002), which indicates that different gene pools had been maintained within the Japanese soybean. In general, the genetic bases among Chinese, Japanese, and US soybean collections are different, and they could be useful sources for increasing the diversity in the breeding process (Cui et al. 2000; Zhou et al. 2000; Zhou et al. 2002). Cui et al. (2000) reported the successful introduction of US soybean germplasm into Chinese breeding programs. Yet, for all the breeding efforts in Japan, even the genetic diversity of Japanese edamame remains unreported. Clarifying the genetic diversity of exotic soybean, including Japanese edamame and Chinese maodou, could be useful for the selection of diverse parents for efficient breeding strategies for local adaptation.

The use of neutral DNA markers has been adapted as a method for understanding genetic diversity and for fingerprinting cultivars (Rongwen et al. 1995), especially when details in pedigree information are lacking. SSR markers have been used to detect genetic diversity and/or important agronomic traits in soybean (e.g. Akkaya et al. 1992, 1995; Cregan et al. 1999; Brown-Guedira et al. 2000) because of their high polymorphic rate and multiple alleles. In soybean SSRs detected the higher expected heterozygosity compared to other genetic markers such as RFLP, RAPD, and AFLP (Powell et al. 1996). It is also found that RFLPs, AFLPs and SSRs in soybean were highly correlated, while SSRs generated hypervariable polymorphisms (Rongwen et al. 1995). Thus, SSR markers were employed as the primary approach to detect differences among accessions and elite edamame lines.

The objectives of this study were to: (1) describe the genetic diversity among Japanese edamame and Chinese maodou and (2) fingerprint WSU breeding lines to distinguish them from the other edamame cultivars for Plant variety protection (PVP) using SSR DNA makers.

Materials and methods

Plant materials

A total of 130 soybean accessions – 107 Japanese edamame, 10 Chinese maodou, a Chinese frozen import (Safeway, Pleasanton, CA), two US edamame cultivar ('Envy' and 'Butterbean'), two US grain soybean, and eight WSU edamame breeding lines were used in this study (Table 1). Most of the Japanese edamame cultivars were purchased in 1999 from Japanese seed distributors. The other Japanese cultivars were collected in Japan by the East Asian Crop Development lab between 1987 and 1990. Ten Chinese maodou accessions were obtained from the USDA soybean germplasm collection in Urbana, Illinois. Maturity information for each cultivar was obtained from the package labels and seed catalogues if available. Maturity class was generally dependent on the daylight length that minimized days to flower in soybean in Japan: Early (A) 11–13 h, Middle (B) 10–12 h and Late (C) 8–10 h.

Table 1. Sample accessions of vegetable (edamame) and other soybean and their traits organized by UPGMA clusters.

Accession	UPGMA Cluster	Seed coat	MG	Seed source	Production	USDA P.I. number	Seed weight
204D	1	Y	N/A	WSU	WA, USA	–	29.25
208B	1	LG	N/A	WSU	WA, USA	–	25.50
209B	1	LG	N/A	WSU	WA, USA	–	24.68
210B	1	LG	N/A	WSU	WA, USA	–	28.50
221C	1	LG	N/A	WSU	WA, USA	–	19.21
245D	1	LG	N/A	WSU	WA, USA	–	22.00
Bearfriend	1	YG	A	Takii	Hokkaido	–	39.30
Fusamidori	1	LG	AA	Nayahara	Hokkaido	–	37.40
GokuwaseFukura	1	YG	AA	Kaneko	Hokkaido	–	31.90
Hamanishiki	1	YG	A	Yokohama	Hokkaido	–	33.50
SapproGokuwaseNo.1	1	YG	AA	Hara	Hokkaido	–	37.65
Sappromidori (SP)	1	YG	A	Snow Brand	Hokkaido	PI 538406	33.27
Shirojishi	1	YG	AA	Takii	Hokkaido	PI 549069	38.70
Tamasudare	1	LG	A	NihonNorin	Hokkaido	–	33.95
Tarafuku	1	N/A	A	Yokohama	Hokkaido	–	39.50
Testy85	1	YG	A	Hara	Hokkaido	–	36.50
Thoya	1	YG	AA	Tokita	N/A	–	42.45
Fukusaya	2	Y	AA	Fukutane	Hokkaido	–	28.70
Gokuwase	2	Y	A	Tohoku	Hokkaido	–	30.00
MonsyuOzaya	2	BR	A	Matsuda	Hokkaido	–	28.40
Natsunoka	2	Y	AA	Sakata	Hokkaido	PI 538404	25.65
Natunoshirabe	2	BR	AA	Sakata	Hokkaido	–	37.50
Okuhara	2	Y	A	Nayahara	Hokkaido	–	33.30
OkuharaHS1	2	Y	A	Hara	Hokkaido	–	30.25
OkuharaWase	2	Y	AA	Takii	Hokkaido	–	35.50
SakigakeHoney	2	BR	AA	Hara	Hokkaido	–	30.00
ShinDenkou	2	Y	AA	Hara	Hokkaido	–	26.90
WaseKaorihime	2	BR	AA	Kyowa	Hokkaido	–	38.50
Aodaizu	3	G	N/A	Oota	Fukushima	–	33.95
Shishiou	3	G	C	Takii	Iwate	–	38.15
Akimaturi	4	YG	B	Hara	Hokkaido	–	38.65
Butterbean	4	LG	(I)	USDA	USA	PI 567194	33.50
Ehigohoney	4	BR	A	Hara	Hokkaido	–	29.75
Ezomidori	4	YG	A	Hara	Hokkaido	PI 549055	31.05
Fuki	4	YG	AA	Takii	N/A	–	35.50
Fukujishi	4	YG	B	Takii	Hokkaido	–	40.75
Fukura	4	YG	A	Kaneko	N/A	–	40.60
Fukusuzu	4	LG	A	Fukutane	Hokkaido	–	28.10
Green75	4	YG	AA	Hara	Hokkaido	–	38.80
Hakucho	4	YG	A	Nayahara	Hokkaido	PI 54057	33.00
HokkaiWase	4	LG	A	Nayahara	Hokkaido	–	35.50
Horoyoi	4	N/A	A	Yokohama	Hokkaido	–	35.70
Houryokusuzunari	4	YG	A	Hara	Hokkaido	–	28.85
Houseki	4	Y	A	Oota	Hokkaido	–	37.50
Housetu	4	Y	A	Marutane	Hokkaido	–	31.50
Ichiriki	4	Y	AA	Kaneko	Hokkaido	PI 549061	46.82
Jusrt75	4	LG	AA	Musashino	N/A	PI 549062	4.67/15
Kachikari No3	4	YG	A (II)	Tokita	Hokkaido	PI 54064	31.58
Kitamidori	4	G	A	Tokita	Hokkaido	–	27.53
Koufuku	4	YG	A	Tohoku	Hokkaido	–	35.00
Kurobee	4	BL	A	Aritaya	Chiba	–	32.50
Kurotarou	4	BL	A	Asashi Ikusyu	Aichi	–	41.00

Table 1. Continued.

Accession	UPGMA Cluster	Seed coat	MG	Seed source	Production	USDA P.I. number	Seed weight
Kyounonatsu	4	YG	A	Marutane	Hokkaido	–	34.15
Lucky Lion	4	YG	N/A	Cascadian	CA, USA	–	
Misono Green	4	Y	A	Snow Brand	Hokkaido	PI 549067	35.47
Natunomai	4	LG	A	Sakata	Gunma	–	30.70
Natunoyosooi	4	BL	A	Sakata	Hokkaido	–	32.00
Natutourai	4	YG	AA	Sakata	Gunma	–	38.60
Nouhime	4	BL	N/A	Takii	Hokkaido	–	32.30
OoharuHakumou	4	Y	A	Kyowa	Hokkaido	–	40.30
Oosodefuri	4	LG	A	Sakata	Hokkaido	–	30.61
Ryokuhou	4	LG	N/A	Marutane	Hokkaido	–	33.75
Ryokusei	4	LG	N/A	Sato	Iwate	–	35.56
Sayakaze	4	YG	AB	Fukutane	Hokkaido	–	37.60
Shiawase	4	BL	A	Matsuda	Hokkaido	–	33.30
ShiretokoOozaya	4	YG	A	Tokita	Hokkaido	–	33.90
Shironomai	4	Y	AB	Sakata	Gunma	PI 538409	36.12
Suzumohakutyou	4	LG	A	Takii	Hokkaido	–	36.50
TaikaiMidori	4	YG	A	Asashi Ikusyu	Hokkaido	–	31.00
TaishoMidori	4	YG	B	Kyowa	Hokkaido	–	43.00
Tamasudare2	4	LG	A	NihonNorin	Hokkaido	–	37.15
Tankurou	4	BL	A	Marutane	Kyoto	–	35.10
Tengamine	4	YG	AA	Sakata	Hokkaido	PI 538410	33.12
ThreeTop	4	LG	A	Asashi Ikusyu	Hokkaido	–	33.00
Wase	4	YG	A	Aritaya	Hokkaido	–	36.50
Wase	4	YG	A	Tohoku	Hokkaido	–	30.50
WaseMidori	4	YG	A	Nayahara	Hokkaido	–	33.05
YukiMidori	4	LG	A	Nayahara	Hokkaido	–	36.50
Yukimusume	4	YG	A	Snow Brand	Hokkaido	PI 549072	33.93
204B	5	N/A	N/A	WSU	WA.USA	–	N/A
213C	5	N/A	N/A	WSU	WA.USA	–	N/A
Ajigen	5	YG	A	Sakata	Hokkaido	–	38.60
Ezohakuhou	5	LG	A	Hara	Hakkaido	–	35.15
HikariKurodaizu	5	BL	N/A	Hara	Hakkaido	–	39.20
Ooguro	5	BL	C	Fukutane	Fukui	–	36.15
Orihime	5	N/A	A	NihonNorin	Hakkaido	–	40.75
Osusume	5	LG	AB	Yokohama	Hokkaido	–	49.46
Sayamusume (SY)	5	YG	A	Snow Brand	Hokkaido	–	48.30
Tsurunoko	5	Y	(IV)	Kyowa	Hokkaido	PI 89128	39.60
Chachamaru	6	BR	AA	Hara	Hakkaido	–	30.95
ChakkiriMusume	6	BR	A	Hara	Hakkaido	–	26.45
Chatarou	6	BR	A	Asashi Ikusyu	Aichi	–	24.50
ChuseiChamame	6	BR	B	Sato	Iwate	–	34.31
Fukunari (Kiyo_hime)	6	BR	A	Takii	Hokkaido	–	26.90
Fusanari_Chamame	6	BR	AA	Aritaya	Chiba	–	28.50
Kouri	6	BR	A	Marutane	Hokkaido	–	29.00
Natunokoe	6	BR	A	Sakata	Hokkaido	–	26.00
Sentya	6	BR	C	Fukutane	Fukui	–	30.10
TadachaMame	6	BR	C	Oota	Yanagata	–	26.45
Tanbaguro	6	BL	C	Aritaya	Hakkaido	–	52.50
Tanbaguro	6	BL	C	Oota	Hyogo	–	60.95
Fukunomai	7	YG	C	Fukutane	Hokkaido	–	40.05
KonishikiJambo	7	YG	C	Fukutane	Fukui	–	39.05
Oshimamidori	7	YG	C	Hara	Hakkaido	–	41.60
Ryokuko	7	YG	C	Sato	Iwate	–	41.04
Safeway (Frozen product)	7	N/A	N/A	Safeway	China	–	N/A

Table 1. Continued.

Accession	UPGMA Cluster	Seed coat	MG	Seed source	Production	USDA P.I. number	Seed weight
Kegon	8	Y	B	Sakata	Saitama	PI 538403	30.75
Soroibumi	8	YG	AB	Yokohama	Hokkaido	–	42.50
Yusuzumi	8	YG	AB	Aritaya	Hokkaido	–	34.00
Yusuzumi	8	YG	B	Sakata	N/A	–	33.50
Cyusei	9	Y	B	Aritaya	Chiba	–	32.00
Mikawashima	9	Y	B	Aritaya	Hokkaido	–	30.75
Chapman (grain soybean)	out	Y	(II)	Public grain	USA	PI 542711	18.00
Chinese1 (CHI1)	out	Y	(V)	USDA	China	PI594810A	1.49/20
Chinese2 (CHI2)	out	Y	(IX)	USDA	China	PI587787	4.03/20
Chinese3 (CHI3)	out	Y	(II)	USDA	China	PI567161	3.68/20
Chinese4 (CHI4)	out	Y	(III)	USDA	China	PI538375	1.40/20
Chinese5 (CHI5)	out	Y	(VII)	USDA	China	PI594808	1.53/20
Chinese6 (CHI6)	out	Y	(IV)	USDA	China	PI594604	1.44/20
Chinese7 (CHI7)	out	Y/BR	(VII)	USDA	China	PI588011E	3.35/20
Chinese8 (CHI8)	out	Y	0	USDA	China	PI567167	3.75/20
Chinese9 (CHI9)	out	Y	(VII)	USDA	China	PI587871	3.60/20
Chinese10 (CHI10)	out	Y	(VI)	USDA	China	PI587581	4.21/20
EchizenMidori	out	G	C	Fukutane	Fukui	–	33.25
Envy	out	G	0	USDA	USA	PI 567180	16.60
Mikawashima	out	Y	B	Sakata	Saitama	–	28.37
Panda	out	BL/G	C	Fukutane	N/A	–	35.50
Ryokuheki	out	N/A	N/A	Kaneko	N/A	–	N/A
SengokuOozaya	out	G	C	NihonNorin	Hokkaido	–	53.20
Spry (grain soybean)	out	Y	(IV)	Public grain	USA	PI553052	17.80

Testa colors are represented; Y: yellow, G: green, LG: light green, YG: yellow green, BR: brown, and BL: black. Maturity class was defined day light length that minimized days to flower in Japan; 11–13 h: early (A), 10–12 h: middle (B), and 8–10 h: late (C). Seed weight was scaled as g/100 seeds unless otherwise indicated. USDA Plant Introduction (P.I.) numbers were denoted if available.

Testa color and seed weight (g/100 seeds) were observed in the lab.

The eight WSU breeding lines were produced with the pedigree method from crosses between the non-shattering US grain soybean cultivar, 'Chapman', and the Japanese edamame cultivar, 'Sayamusume', and backcrossed with either 'Sapporomidori' or 'Sayamusume' (Snow Brand Seed Co., Ltd.). Field trials and selections for superior inbred lines were conducted at the WSU's Irrigated Agriculture Research and Extension Center (IAREC, Prosser, WA).

The plant materials were grown in the WSU greenhouse for DNA isolation, except for one Chinese accession, whose DNA was extracted from commercial frozen product imported from China. The DNA was isolated from approximately 2 g of fresh young leaf tissue from each plant by modified CTAB method (Murray and Thompson 1980). Isolated DNA was diluted with 200μL of TE buffer to concentrations of approximately 500–1200 ng/μL.

Genotyping by SSRs

Each isolated DNA sample was diluted with sterile distilled water (1:20 v:v) for PCR. SSR primer sets were selected from each of 20 genetic linkage groups developed by Cregan et al. (1999) to detect unbiased diversity. Three SSR primers detected bands too faint to score or generated unspecific bands, and seventeen primers were used in this study (Table 2). The PCR procedure followed Cregan and Quigley (1997). The PCR reagents contained 3 μL of diluted DNA, 50 mM of KCl, 10 mM of Tris–HCl (pH 9.0), 0.1% of Triton X-100, 1.5 mM of $MgCl_2$, 0.15 mM of each nucleotide and 1 unit of Taq DNA polymerase. PCR cycling consisted of 30 cycles of 30 s denaturation at 94 °C, 30 s annealing at a range of 47–55 °C and 30 s extension at 68 °C, following 2 m pre-denaturation at 94 °C on a Perkin Elmer Gene-Amp PCR system 9600. SSR primer sets required an

Table 2. SSR primers used in this study and summary of genetic diversity.

Linkage group[a]	Primer	Number of alleles	Gene diveristy
MLG A1	Satt042	6	0.410
MLG A2	Sat_040	6	0.568
MLG B1	Sct_026	4	0.461
MLG B2	Satt556	12	0.625
MLG C2	Satt079	8	0.514
MLG D1a + Q	Satt071	2	0.150
MLG D1b + W	Sat_135	3	0.622
MLG D2	Satt458	10	0.762
MLG F	Satt030	9	0.688
MLG G	Satt012	4	0.350
MLG H	Satt279	8	0.664
MLG I	Satt270	6	0.532
MLG J	Satt596	7	0.618
MLG K	Satt544	11	0.841
MLG L	Satt182	8	0.496
MLG M	Satt540	8	0.541
MLG N	Satt530	7	0.460
Average		7	0.547

[a]Linkage map groups are adapted from Cregan et al. (1999).

annealing temperature of either 47, 50 or 55 °C to detect clear specific bands. PCR products were separated on a denaturing DNA sequencing gel containing 6% of polyacrylamide, 8 mM of urea and 1×TBE at 60 W constant power for 2–3 h. All gels were visualized by silver stain (Bassam et al. 1991).

Statistical analysis

Diversity values for each locus were calculated using Nei's genetic diversity index $h = 1 - p^2_i$, where p^2_i is the frequency of the *i th* allele. All SSRs detected at least one polymorphism among the entire collection of accessions. All bands were visually counted twice, and genetic similarity was calculated by Jaccard's coefficient (Jaccard 1908), $P = a/(n - d)$, where '*a*' is the bands present in both compared genotypes, '*n*' is the total number of polymorphic bands and '*d*' is the bands absent in both compared genotypes. A dendrogram was generated from the sequential, agglomerative, hierarchical, and nested (SAHN) clustering method using the unweighted pair-group method, arithmetic average (UPGMA) (Sneath and Sokal 1973) by NTSYSpc (Rohlf 2000). The multidimensional scaling was obtained by using a distance matrix created by %DISTANCE of PC SAS MACRO using Jaccard's coefficient subjected to the MDS procedure of PC SAS. The ABSOLUTE option was used in the MDS procedure (SAS Institute Inc. 2000).

Results

Of the 20 SSR primers applied in this study, 17 SSRs detected polymorphisms. The 17 SSRs detected a total of 122 alleles in the 130 accessions. The average numbers of alleles and gene diversity per locus for all accessions were 7.0 in a range of 2 to 12 and 0.547 in a range of 0.150 to 0.841, respectively (Table 2). The allele diversity among all the lines in this study was generally lower than that reported by Cregan et al. (1999) but higher at Satt544. Twenty seven alleles were unique in the 107 Japanese edamame accessions, while 24 alleles were unique from the 10 Chinese landraces (Table 3).

The average similarity of estimation was 0.309 within all accessions, 0.357 within Japanese edamame, and 0.032 within Chinese maodou. The genetic similarity was in a range of 1.00 to 0.00 among all accessions, which means that some accessions appear identical, while some accessions appear to be totally different genotypes based on the SSRs. A total of 99 accessions had distinguishable fingerprints, yet 31 Japanese cultivars could not be distinguished from one or more of the

Table 3. Summary of allele distribution among sources.

	Total number of accession	Total number of alleles	Number of unique alleles
Japanese vegetable soybean	107	89	27
Chinese maodou	10	76	24
Frozen product from China	1	18	0
US vegetable soybean	2	30	0
US gain soybean	2	27	2
WSU breeding lines	8	35	0
Total	130	122	–

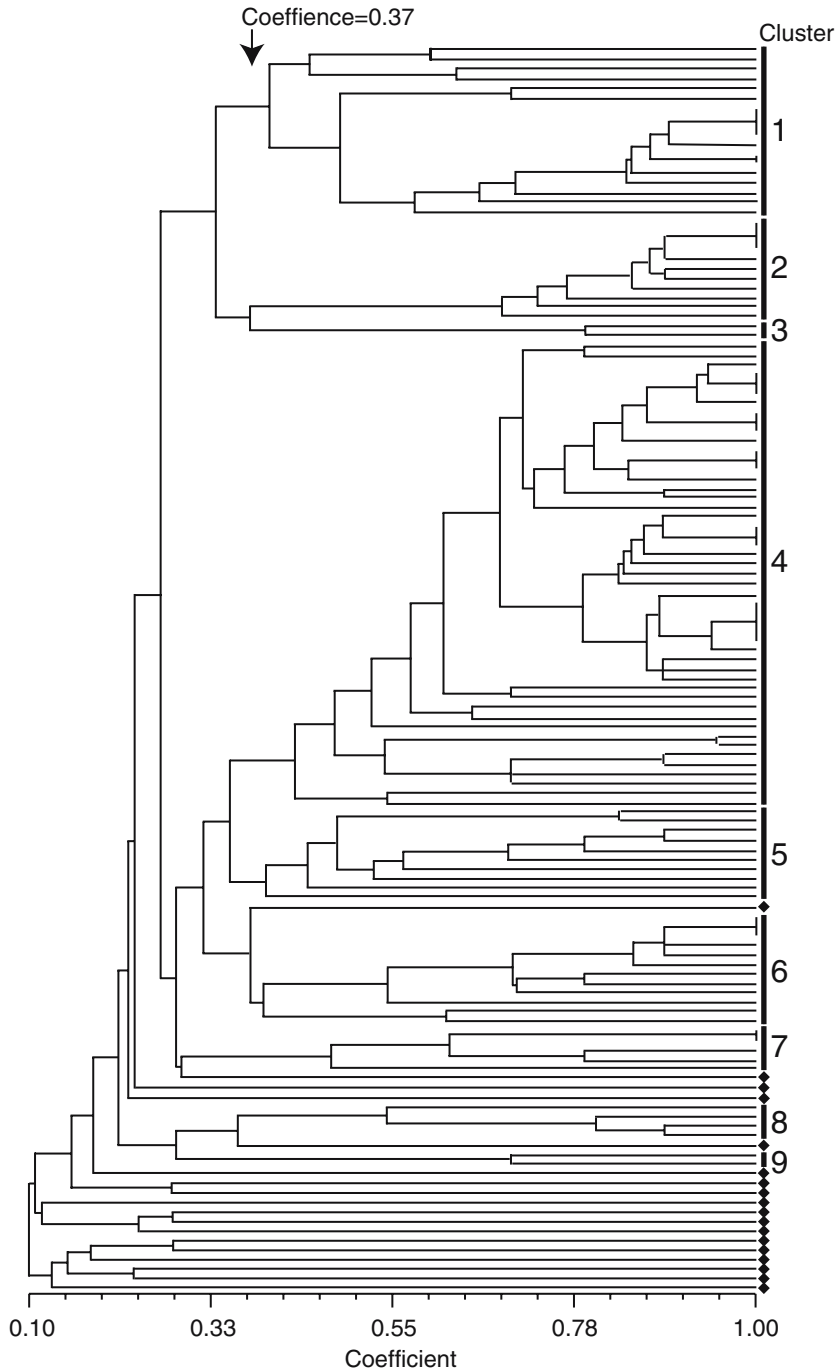

Figure 1. Dendrogram of genetic relationship among soybean accessions based on 17 unlinked SSR markers. Clusters were defined at Jaccard's coefficient 0.37. Numbers on the right side of the dendrogram are indicated cluster numbers, which are listed in Table 1. Filled diamonds are indicated outliers.

other Japanese cultivars by the use of the 17 SSRs. All WSU breeding lines were genetically distinguishable from the others.

The dendrogram produced by the UPGMA algorithm for clustering of the accessions is shown in Figure 1. At 0.37 of coefficient, nine clusters

(Cluster 1 to 9) and 18 outliers were apparent. Most Japanese edamame cultivars fell into one of the nine clusters, and all WSU breeding lines fell into either Cluster 1 or 5 along with either one of their Japanese edamame parents. Cluster 1 was comprised of six WSU lines, US edamame cultivar and 11 Japanese cultivars including one of the edamame parents for the WSU lines, 'Sapporomidori.' Some Japanese accessions demonstrated no dissimilarity, while the six WSU breeding lines were genetically distinguishable in this cluster (Table 1 and Figure 1). Cluster 2 contained 11 Japanese cultivars, all with very early or early maturity. Cluster 3 contained only two Japanese accessions that both have dark green testa. Cluster 4 was the largest cluster with 49 cultivars. One of the US edamame, 'Butterbean,' and 48 Japanese edamame cultivars fell into this cluster. Cluster 5 contained 2 WSU breeding lines and 8 edamame cultivars, including one of the parents for the WSU breeding lines, 'Sayamusume.' Ten brown testa and two black testa Japanese edamame cultivars fell into Cluster 6. Cluster 7 contains 4 Japanese cultivars and the frozen edamame imported from China (Safeway Inc.). Cluster 8 consisted of four Japanese accessions. Cluster 9 had two middle maturity Japanese edamame cultivars, 'Chusei' and 'Mikawashima'. Eighteen accessions did not belong to any clusters and are therefore labeled as recognized outliers. The outliers include 10 Chinese maodou accessions, the two US grain soybean, one US edamame cultivars, and five Japanese edamame cultivars.

The multidimensional scoring (MDS) employed three-dimensional analysis. The plot of Dimensions 1×2 of MDS reflects the results of the UPGMA clustering (Figure 2). The plot of Dimensions 1×3 of MDS more clearly describes outliers surrounding Japanese edamame accessions (Figure 3) compared to the plot of Dimensions 1×2. These multidimensional plots show that many Japanese edamame cultivars have one core of genetic diversity, as illustrated in Cluster 4 of the UPGMA dendrogram (Figure 1).

Discussion

Edamame genetic diversity

The SSRs successfully distinguished cultivars within the narrow genetic diversity among edamame cultivars. However, 31 Japanese cultivars are genetically indistinguishable from one or more of their members based on these SSRs. These SSR identical cultivars may share parents and/or pedigrees. Sharing elite parents and pedigrees is likely due to a strong breeding preference to cross elite with elite to maintain the traits for edamame use, selected for the most specific quality and external appearance requirements demanded by the markets among all soybean use categories. There are popular shared cultivars, such as 'Mikawashima.' Edamame development in Japan probably began with local selection of a suitable line for edamame use from among soybean landraces, which could result in fewer genetic gene pools than others. This origin provided parts of the diversity within today's edamame cultivars.

Even though only 10 accessions from Chinese maodou landraces were included in this study, the similarity within these Chinese accessions was remarkably low (0.032) compared to that within Japanese accessions (0.357). The Chinese accessions also had private alleles, which indicated that Chinese accessions are very diverse and could be a source of new alleles genetically different from Japanese accessions. A similar result was previously reported in that Japanese and Korean grain soybean germplasm were found to be genetically dissimilar to Chinese germplasm (Abe et al. 2003). Based on the coefficient of parentage analysis, Zhou et al. (2000) reported that only 5% of the genes from within Japanese soybean released during 1950–1988 were from Chinese soybean germplasm and that only 2% were from US soybean germplasm. While Chinese breeders strongly avoid mating related pedigree and strive to introduce new germplasm from the US (Cui et al. 2000), Japanese edamame soybean cultivars tend to be developed from hybridizations within the same genetic pools.

Japanese edamame have one core group showing similar SSR diversity using multidimensional scoring (Figures 2 and 3). They also have a unique genetic base, since they are genetically distinguishable from the US grain soybean and Chinese maodou landraces. This agrees with the coefficient of paternity analysis of Japanese soybean by Zhou et al. (2000) and Abe et al. (2003). This unique base implies a long history of Japanese soybean cultivation and edamame improvement after edamame's initial introduction from China.

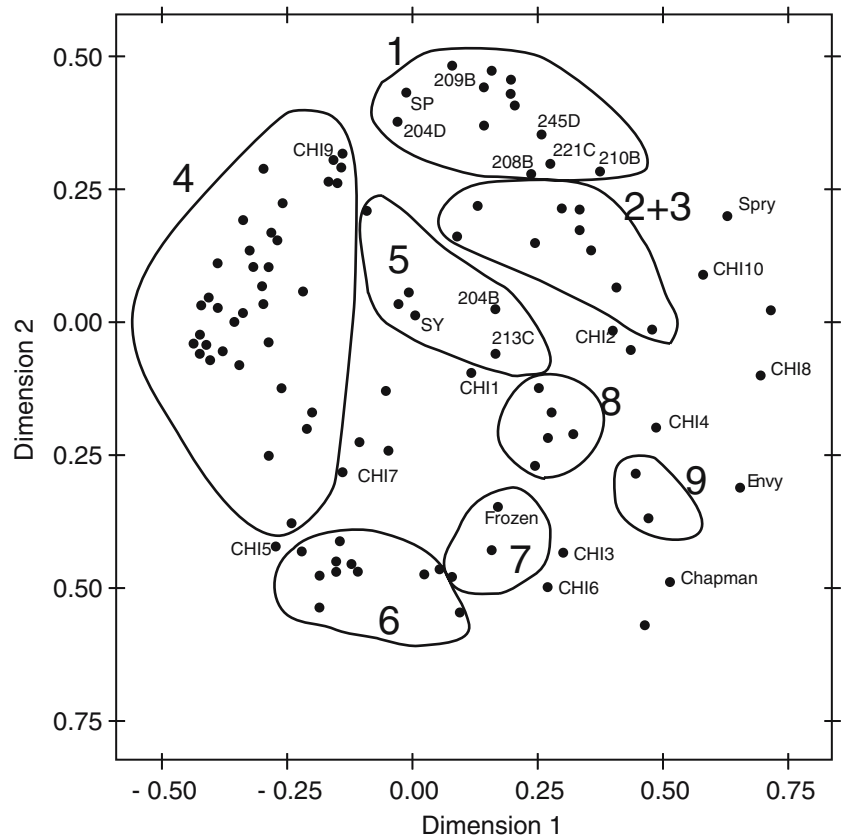

Figure 2. The multidimensional scaling plots of Dimension 1 and 2. Plot points are indicated by dots and represent adjoining accession codes, which are listed in Table 1.

The clustered edamame cultivars

Clustering analysis reflected testa color and maturity classification used for soybean cultivars. Cluster 3 contained two dark green testa edamame, while all 12 accessions in Cluster 6 had either brown or black testa regardless of their different maturity classes. For most of maturity classes, very early or early maturity classes fell into Clusters 1, 2 and 4, Cluster 7 was a group of late maturity cultivars, and Clusters 8 and 9 contained middle maturity cultivars. This suggested that pedigrees were shared within the clusters, since SSR markers are supposed neutral to selection.

All 12 Japanese edamame cultivars belonging to Cluster 6 are brown or black seed coat edamame, such as 'Tadachamame' and 'Tambaguro,' and are popular landraces. 'Tadachamame' and 'Tanbaguro' are especially well known as good tasting edamame. Two accessions labeled 'Tanbaguro,' one harvested from Hokkaido (lat. 42–43° N) and one from Kyoto (lat. 35° N), showed a similarity of only 0.62. This may have resulted from genetic drift in heterogeneous landraces. These brown edamame cultivars belonging to Cluster 6 probably came from related parents.

Cluster 9 and outliners contain two 'Mikawashima,' which have middle maturity. This cultivar may have acquired diversity when maintained in a different seed source and grown in a different production area. In this study, several pairs of accessions with the same name had very similar genotypes, as measured by the SSR alleles. Although these pairs of accessions have the same name, SSR fingerprinting indicated that they are slightly different from each other. This difference may be due to a genetic shift caused by environmental factors affecting selection in

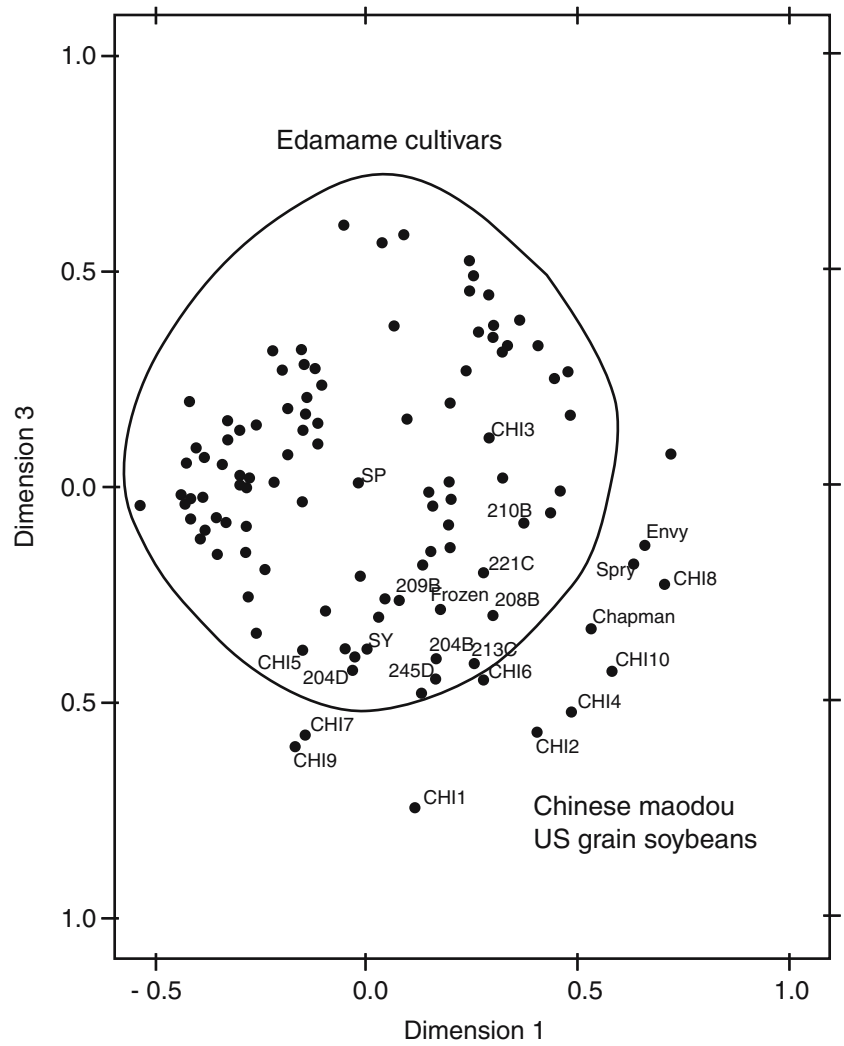

Figure 3. The multidimensional scaling plots of Dimension 1 and 3. Plot points are indicated by dots and represent adjoining accession codes, which are listed in Table 1.

different production areas, mixed seed lots, and/or a high mutation rate in soybean as reported by Diwan and Cregan (1997).

The remaining accessions in our study clustered at >0.37 of Jaccard coefficient and are recognized as outliers. Outlier accessions included all 10 Chinese maodou landraces, but excluded a frozen product imported from China. The frozen Chinese export product is probably of Japanese parentage. The 10 Chinese landraces and the US grain soybean are morphologically, geographically, and genetically distinguishable from Japanese edamame.

WSU breeding lines and implication for edamame breeding

DNA fingerprinting was applied to the WSU breeding lines in order to determine their divergence from other soybean cultivars. Current WSU breeding lines meet PVP, since all WSU breeding lines were genetically distinguishable from all other accessions used in this study. Six of the eight WSU lines were in Cluster 1 along with one of the backcross parent, and another two lines were in Cluster 5 with their edamame parent (the backcross parent was same as the edamame primary parent; Table 4).

Table 4. Pedigree information of WSU edamame breeding lines with resistance to pod shattering.

Lines	Pedigree1	Pedigree2	Backcross
204B	Sayamusume	Chapman	Sayamusume
204D	Sayamusume	Chapman	Sapporomidori
208B	Sayamusume	Chapman	Sapporomidori
209B	Sayamusume	Chapman	Sapporomidori
210B	Sayamusume	Chapman	Sapporomidori
211A	Sayamusume	Chapman	Sayamusume
213C	Sayamusume	Chapman	Sayamusume
221C	Sayamusume	Chapman	Sapporomidori
245D	Sayamusume	Chapman	Sapporomidori

Backcross was conducted at least twice with same backcross parents, and this influenced genetic diversity in WSU breeding lines. All WSU breeding lines had at least 0.1 (e.g. 204B) of coefficient from their grain soybean parent, 'Chapman.' This indicates that the breeding lines are recovering most of the desired edamame cagronomic traits but still retain the non pod-shattering, a trait from the US grain soybean parent. However, although WSU lines were clustered with rather large seed-size edamame cultivars, the average seed of WSU lines at the tested generation was smaller than the average Japanese edamame cultivars, and the variation in seed size is still high (data not shown). The broad sense heritability for seed weight of edamame soybean was reported to be 87.1% (Wasee et al. 1992). Further intense selection should be applied to achieve favorable seed size. Moreover, important goals of edamame breeding are not only large seed size, but also taste and flavor, early maturity for marketing (Konovsky et al. 1994), and resistance to pests (Kraemer et al. 1994).

The dendrogram produced by this study reveals genetic structure among the accessions and will be useful for the selection of parents for future edamame breeding programs. Chinese, US, Canadian, and Korean soybean could be good sources of germplasm to broaden the genetic base, though careful selection from non-elite germplasm will be necessary, as edamame requires a very high-quality product to be competitive in the global edamame market.

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